1ST INTERNATIONAL CONFERENCE ON

Ph+Leukemias

Bologna, Royal Hotel Carlton **September 29-30, 2025**

MUTATIONS IN BCR::ABL1 AND BEYOND

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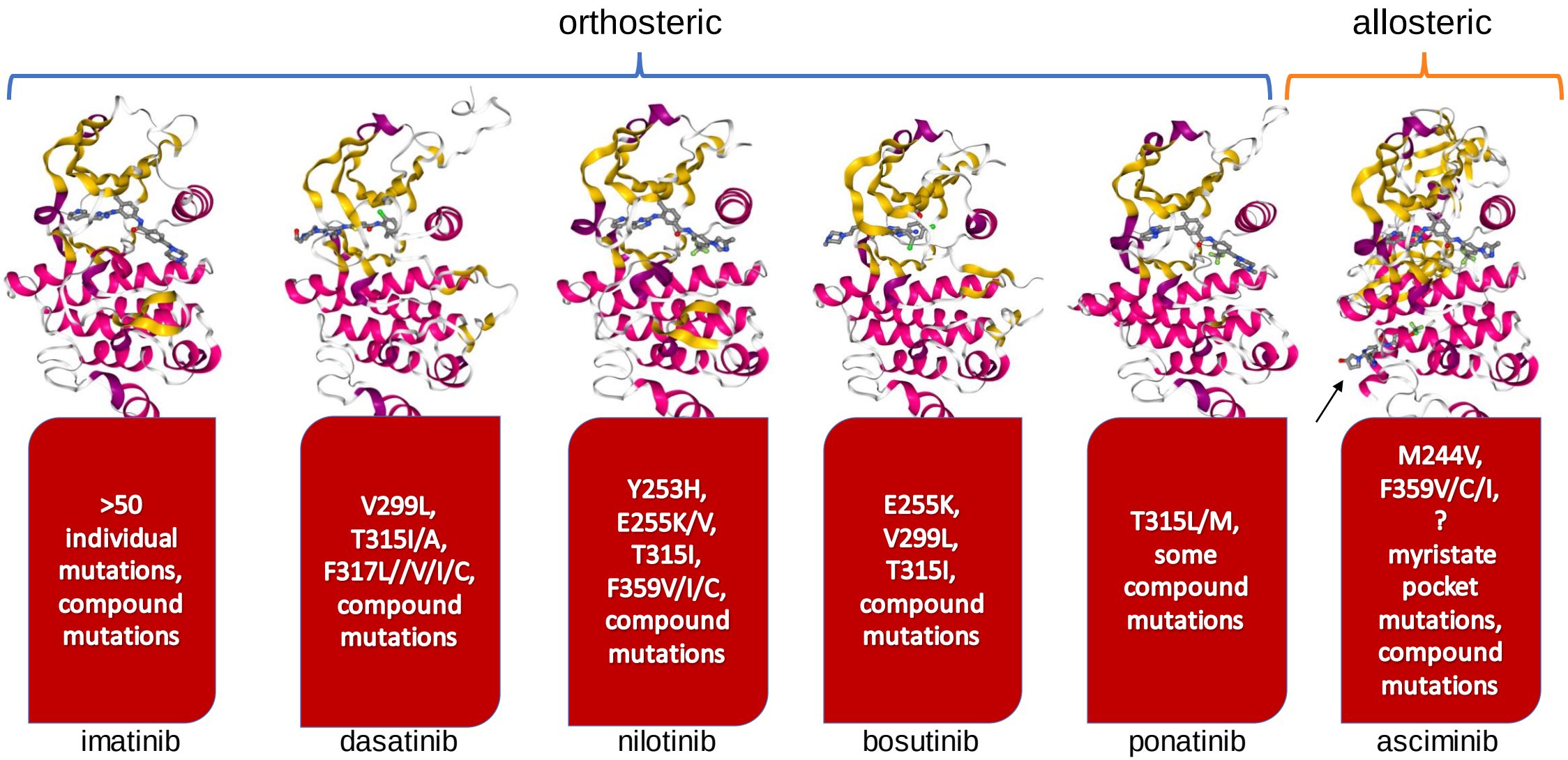
UNIVERSITY OF BOLOGNA

Disclosures for SIMONA SOVERINI

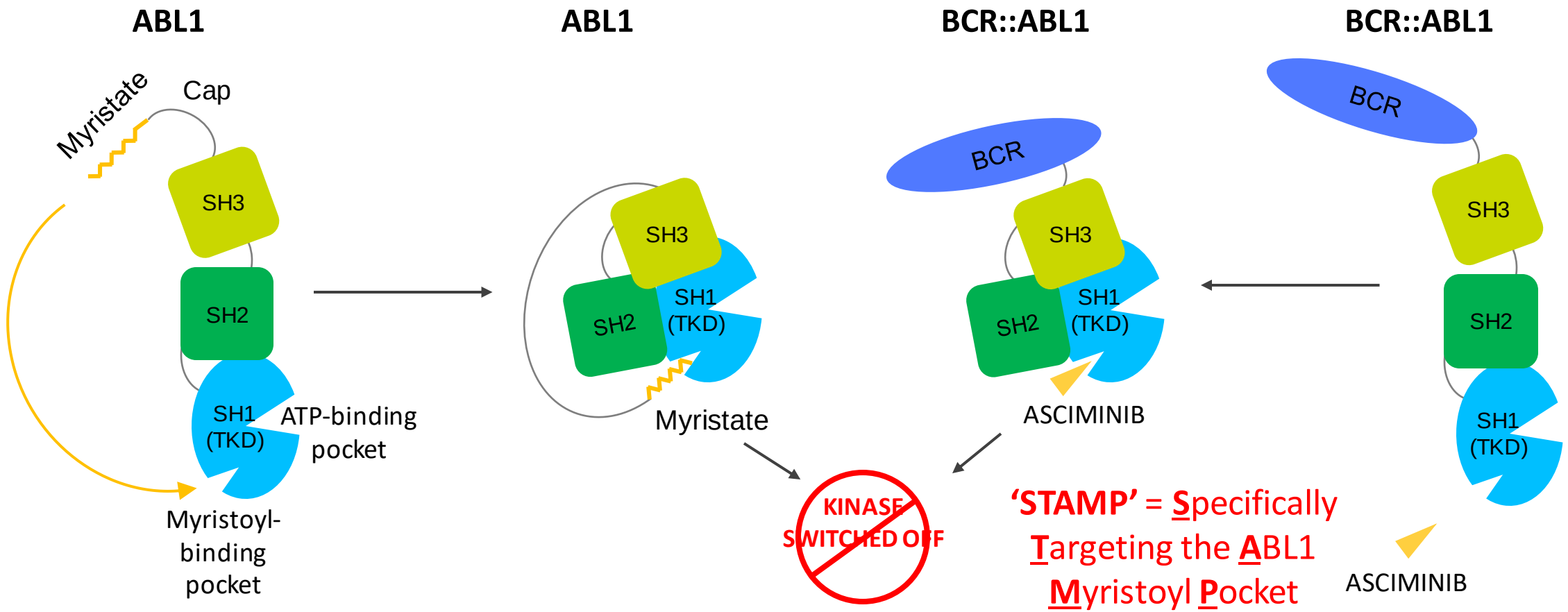
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Incyte Biosciences			X				
Novartis			X			X	

Mutations in CML: the past, the present, the future

Each TKI has mutational Achilles heels



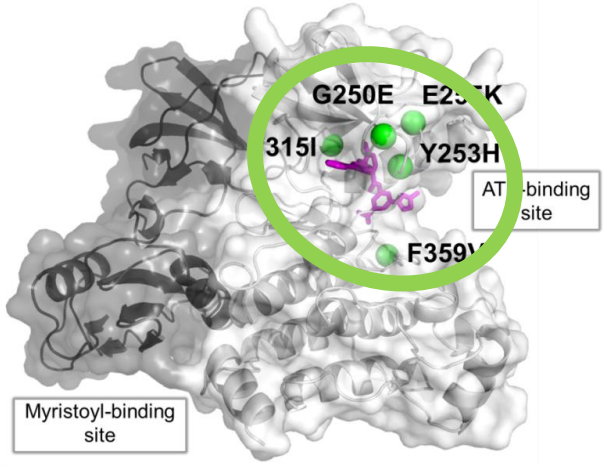
Mechanism of action of asciminib (and other STAMP inhibitors)



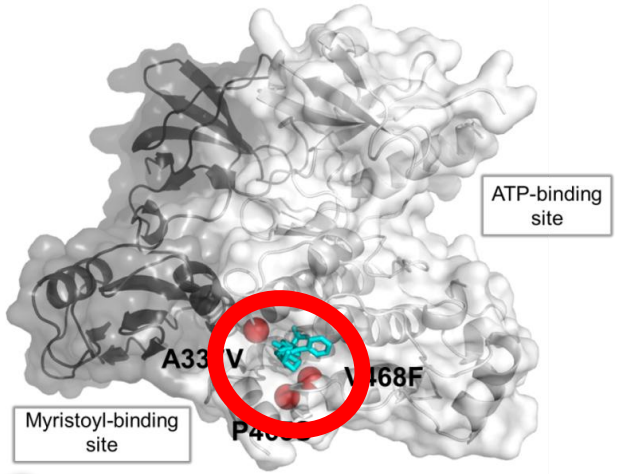
Myristate (and asciminib) binding induce a conformational change and stabilize the closed (inactive) conformation of the kinase

Zhang et al, Nature 2010; Wylie et al, Nature 2017

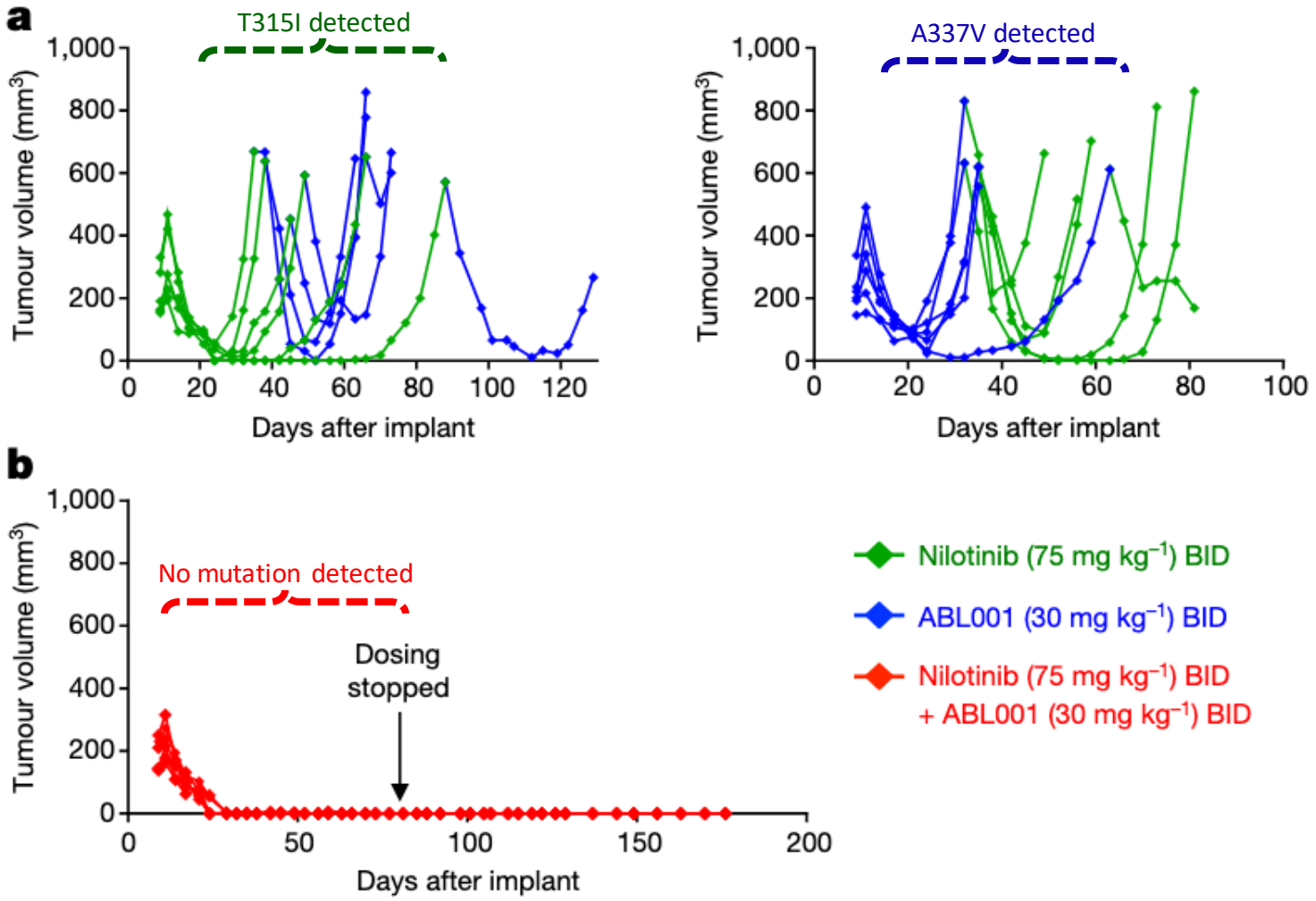
Mutation profiles of asciminib and nilotinib anticipated to be non-overlapping



Nilotinib resistance mutations

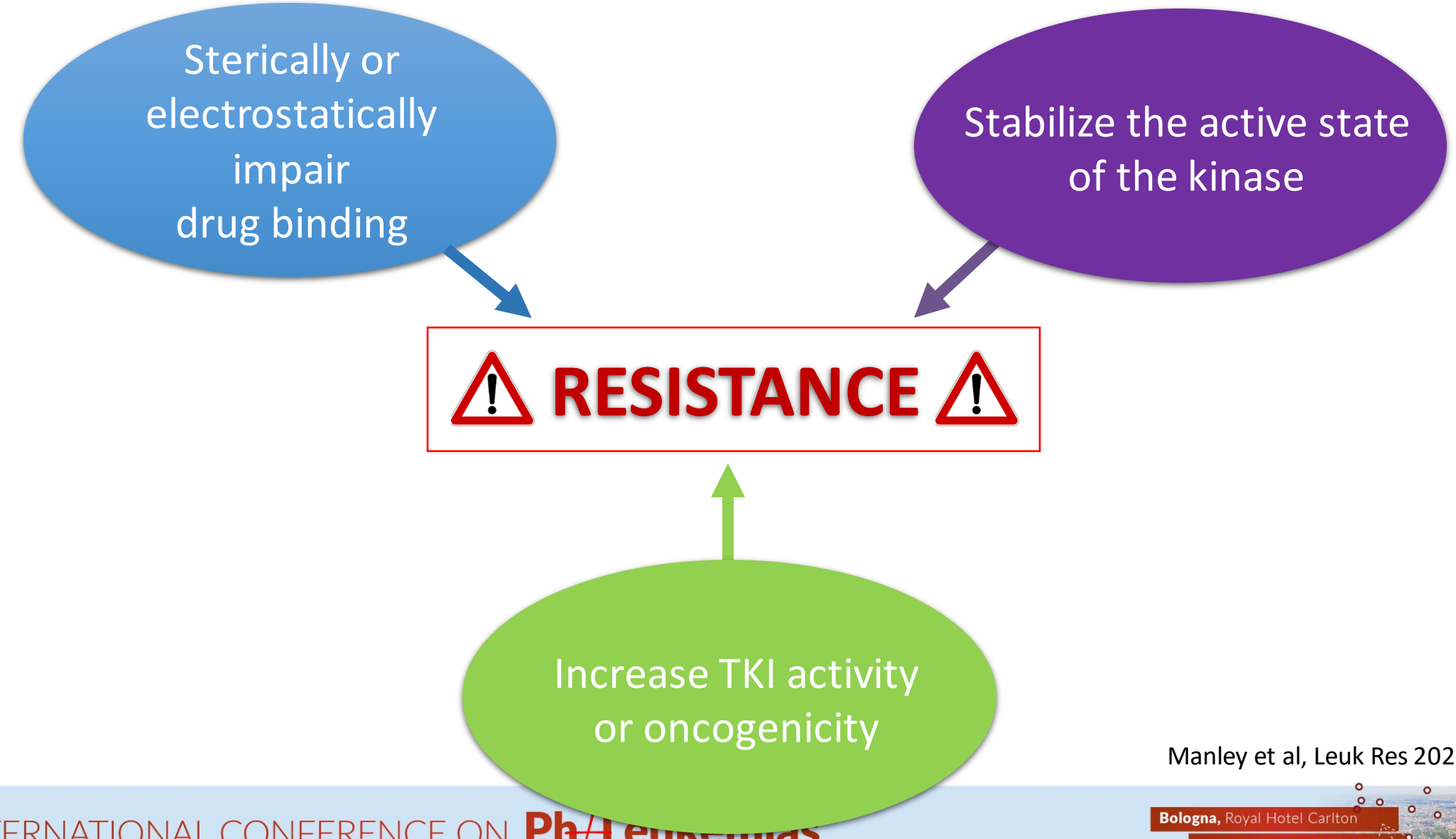


Asciminib resistance mutations



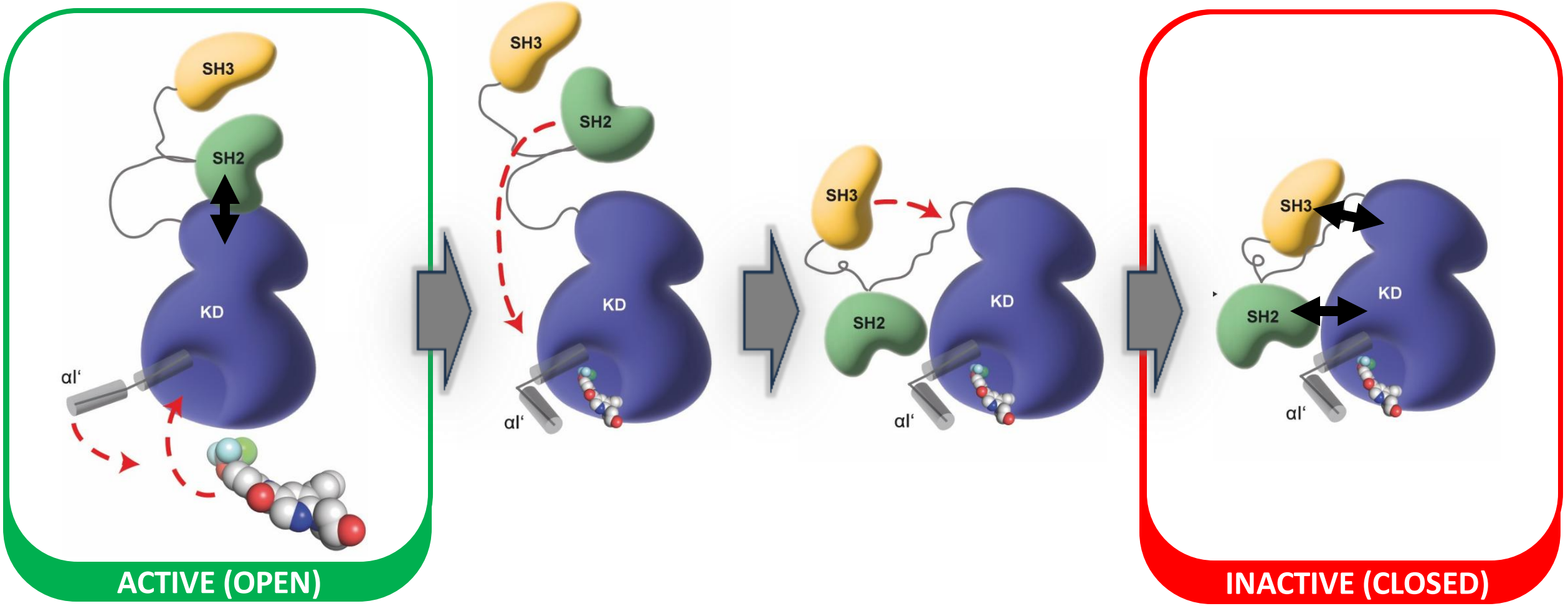
Wylie et al, Nature 2017

How BCR::ABL1 mutations may trigger resistance



Manley et al, Leuk Res 2020

Transition from active to inactive (BCR::)ABL1 requires a major structural reorganization



SH2 ↔ N-lobe

SH2 ↔ C-lobe

SH3 ↔ SH2-CD linker ↔ N-lobe

Modified from Leske and Hantschel, Leukemia 2024

Transition from active to inactive (BCR::)ABL1 requires a major structural reorganization

SH2 ↔ N-lobe

SH2 ↔ C-lobe

SH3 ↔ SH2-CD linker ↔ N-lobe

Modified from Leske and Hantschel, Leukemia 2024

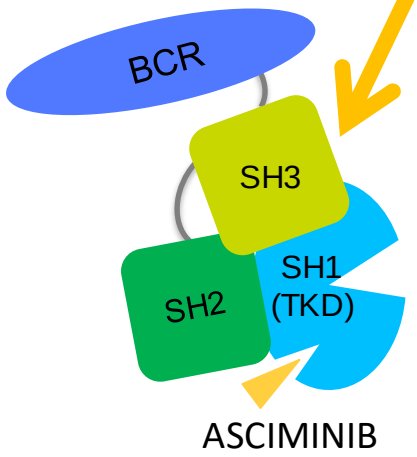
Mutational vulnerabilities of asciminib extend beyond myristate pocket

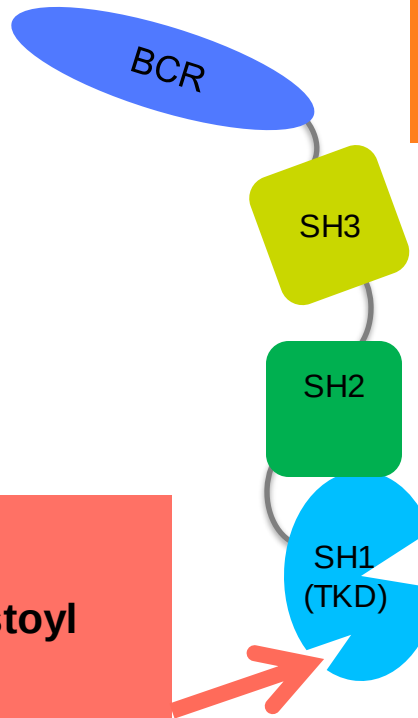
Y115N

Interface between SH3 and KD

→

STERIC CLASH IMPAIRS CLAMPING OF SH3 TO SH1





M244V

N-lobe of the KD

→

STABILIZATION OF THE ACTIVE CONFORMATION

A337T/V

In or near the myristoyl pocket

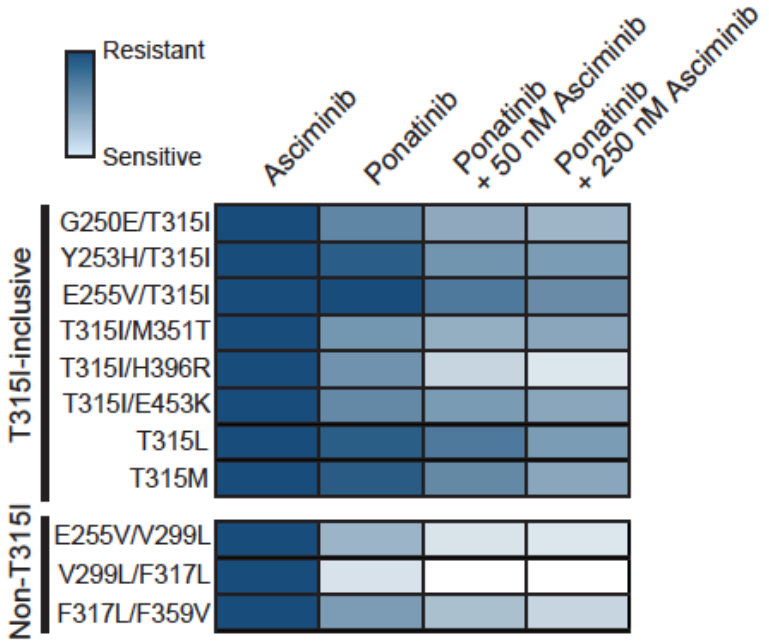
→

ASCIMINIB BINDING IMPAIRED

Leyte-Vidal et al, Blood 2024; Cullot et al, Ann Hematol 2025; Hantschel & Leske, personal comm

Asciminib and compound mutations

- Compound mutations are ≥2 mutations on the same BCR::ABL1 allele
- Compound mutations result from sequential use of narrow-spectrum TKIs (TKIs vulnerable to resistance from single point mutations--> imatinib and 2GTKIs)
- Preclinical (Eide et al, Cancer Cell 2019) and clinical data (Eide et al, Cancer Cell 2019; Kockerols et al, Haematologica 2023; Cortes et al, Leukemia 2024; Chanut et al, Blood 2025) in CML and Ph+ ALL patients indicate compound mutations as frequent responsible of resistance to asciminib in patients already harboring a TKD mutation
- Emergence of compound mutations as a mechanism of relapse was particularly frequent in the phase 1 in T315I-positive patients (Cortes et al, Leukemia 2024)
- PREVENTING RATHER THAN OVERCOMING COMPOUND MUTATIONS REMAINS FUNDAMENTAL!

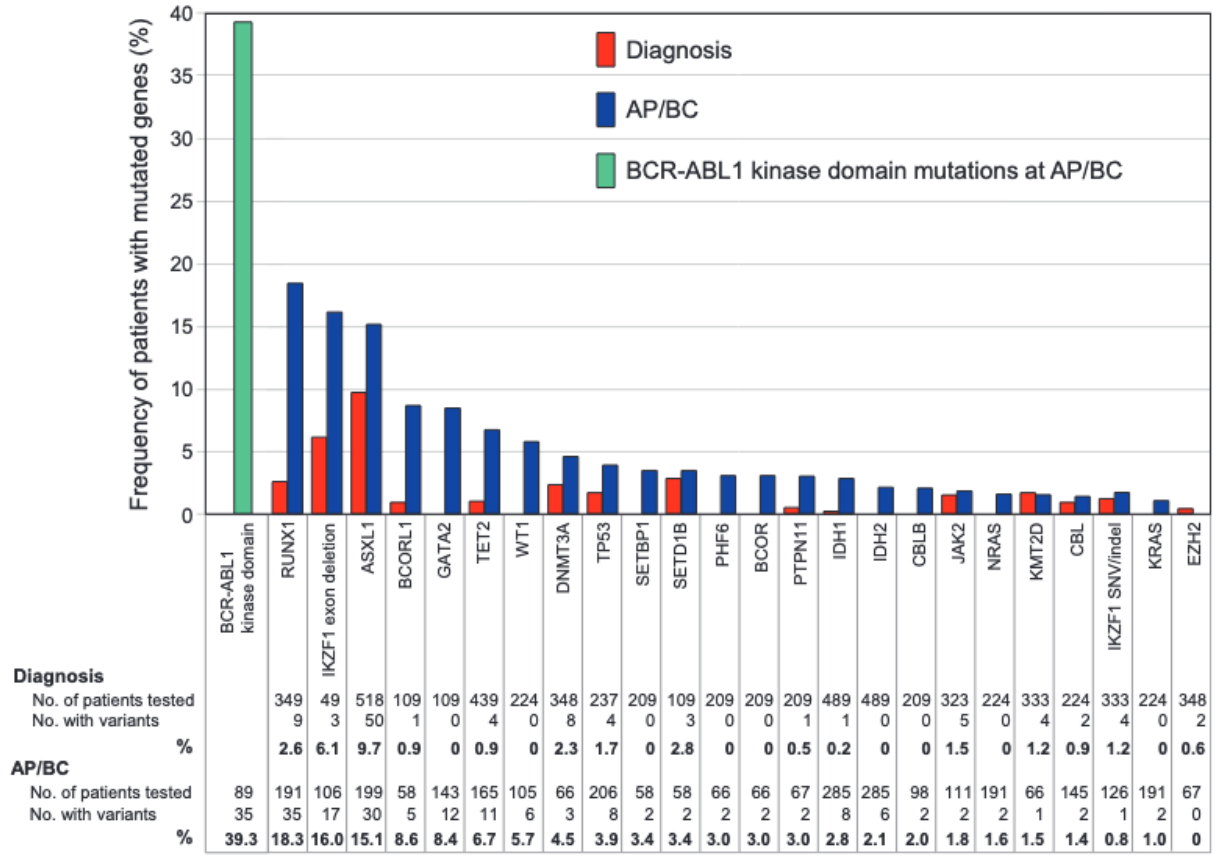


Eide et al, Cancer Cell 2019

Mutations in CML: the past, the present, the future

The mutation landscape of CML beyond BCR::ABL1

- In recent years, wider and wider application of targeted NGS and WES/WGS has revelead that CP CML is not so genetically homogeneous as previously thought!
- Mutations in cancer genes (CG) reported in 16%-20% of newly diagnosed CP CML patients
- Most frequent: ASXL1 (~7-10%)

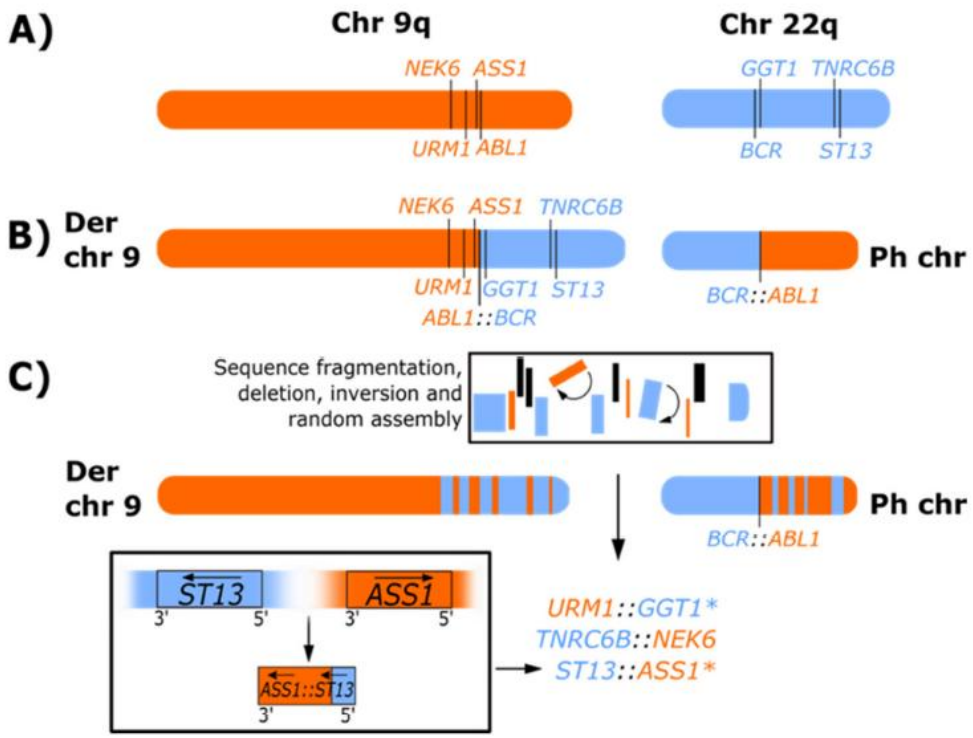


	BCR-ABL1 kinase domain	RUNX1	IKZF1 exon deletion	ASXL1	BCORL1	GATA2	TET2	WT1	DNMT3A	TP53	SETBP1	SETD1B	PHF6	BCOR	PTPN11	IDH1	IDH2	CBLB	JAK2	NRAS	KMT2D	CBL	IKZF1 SNV/indel	KRAS	EZH2
Diagnosis																									
No. of patients tested		349	49	518	109	109	439	224	348	237	209	109	209	209	209	489	489	209	323	224	333	224	333	224	348
No. with variants		9	3	50	1	0	4	0	8	4	0	3	0	0	1	1	0	0	5	0	4	2	4	0	2
%		**2.6**	**6.1**	**9.7**	**0.9**	**0**	**0.9**	**0**	**2.3**	**1.7**	**0**	**2.8**	**0**	**0**	**0.5**	**0.2**	**0**	**0**	**1.5**	**0**	**1.2**	**0.9**	**1.2**	**0**	**0.6**
AP/BC																									
No. of patients tested	89	191	106	199	58	143	165	105	66	206	58	58	66	66	67	285	285	98	111	191	66	145	126	191	67
No. with variants	35	35	17	30	5	12	11	6	3	8	2	2	2	2	2	8	6	2	2	2	1	2	1	2	0
%	**39.3**	**18.3**	**16.0**	**15.1**	**8.6**	**8.4**	**6.7**	**5.7**	**4.5**	**3.9**	**3.4**	**3.4**	**3.0**	**3.0**	**3.0**	**2.8**	**2.1**	**2.0**	**1.8**	**1.6**	**1.5**	**1.4**	**0.8**	**1.0**	**0**

Branford et al, Leukemia 2019

«Ph-associated rearrangements» are novel recurrent genomic alterations in newly diagnosed CML

- Reported in 18% of CP patients at diagnosis
- structural variants represented by aberrant fusions formed at the time of the t(9;22) translocation, involving rearrangement of genes or sequences on the translocated chromosomes
- characterized by sequence fragmentation and imperfect reassembly, multiple deletions, inversions, likely resulting from genomic ‘shattering’ and attempted realignment

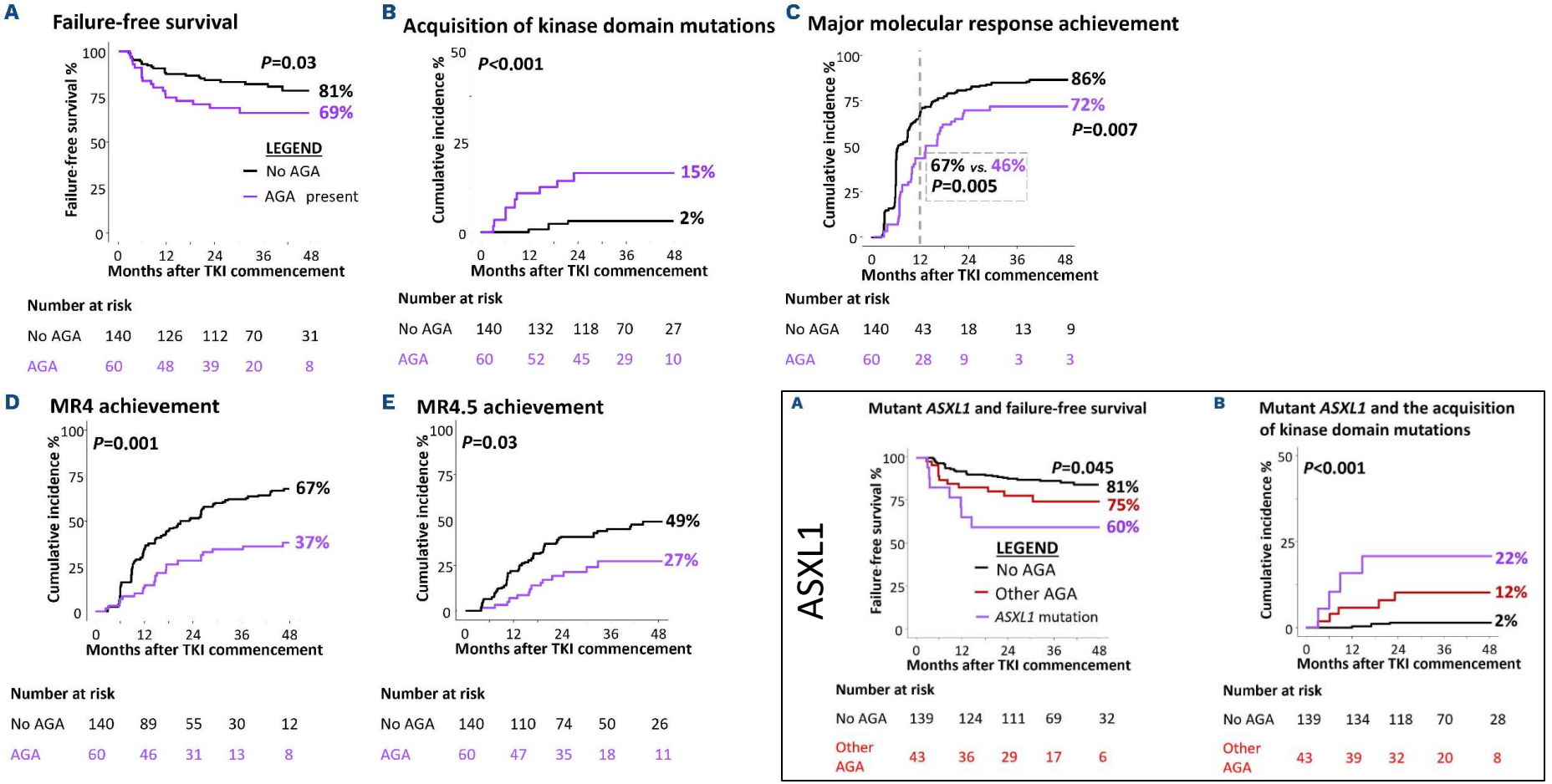


Branford et al, Blood 2018; Fernandes et al, Cancers 2022

Additional genetic abnormalities (AGA) at diagnosis are associated with lower response rates to imatinib

TIDEL II study: CML patients (n=210); 1st-line imatinib 600 mg/d with proactive dose escalation or switch to nilotinib for lack of achievement of time-dependent molecular milestones

AGA = cancer gene variants + Ph-associated rearrangements



Shanmuganathan et al. Haematologica 2023

2GTKIs or asciminib first-line might not overcome the negative impact of ASXL1 mutations

315 CP CML patients consecutively treated in Australiasian studies with frontline more potent BCR::ABL1 inhibitors:

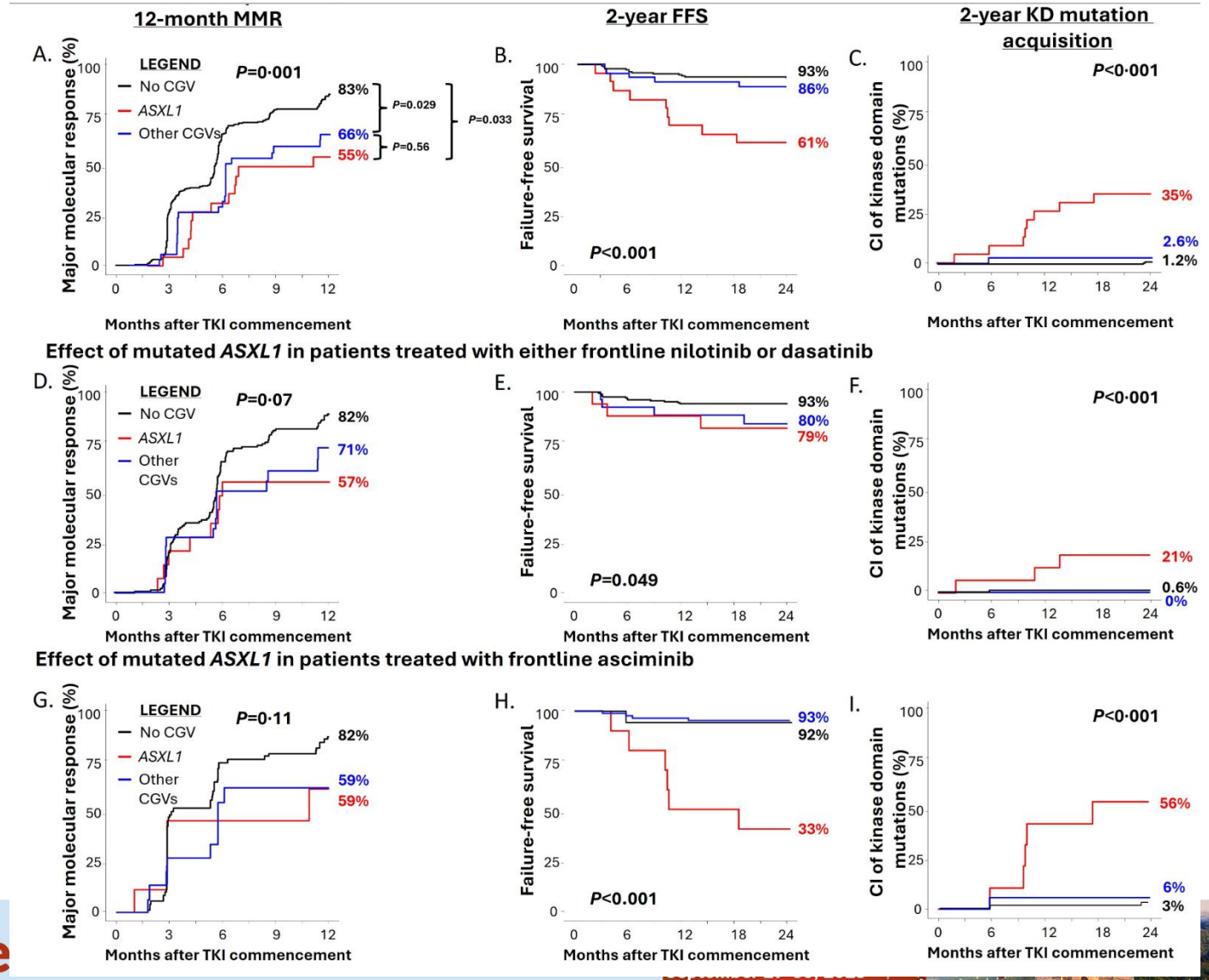
- 2G-TKIs, n=216:

  Nilotinib: ENESTxtnd or CML11 (PINNACLE),

  Dasatinib: CML12 (DIRECT)

- Asciminib, n=99: CML13 (ASCEND)

While the negative impact of Ph-associated rearrangements was overcome by more potent inhibitors, patients with cancer gene mutations continued to have inferior outcomes. This was largely attributable to patients with *ASXL1* variants



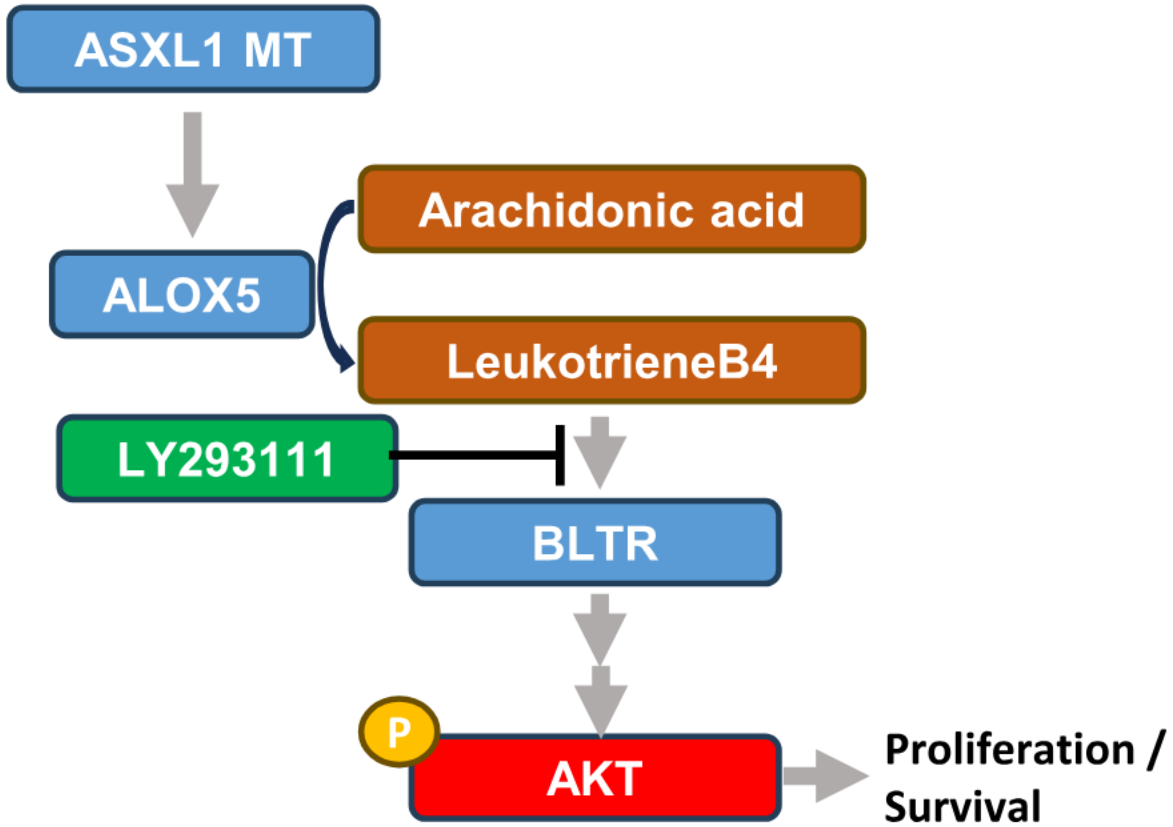
Shanmuganathan et al, Blood 2025

Why do cancer gene/ASXL1 mutations may associate with reduced TKI efficacy?

Do they just reflect greater genetic instability, maybe greater genomic complexity / mutation load, hence more aggressive / high risk disease?

Do they play a genuine pathogenetic role by activating oncogenic processes additional to BCR::ABL1?

A possible pathogenetic role for ASXL1

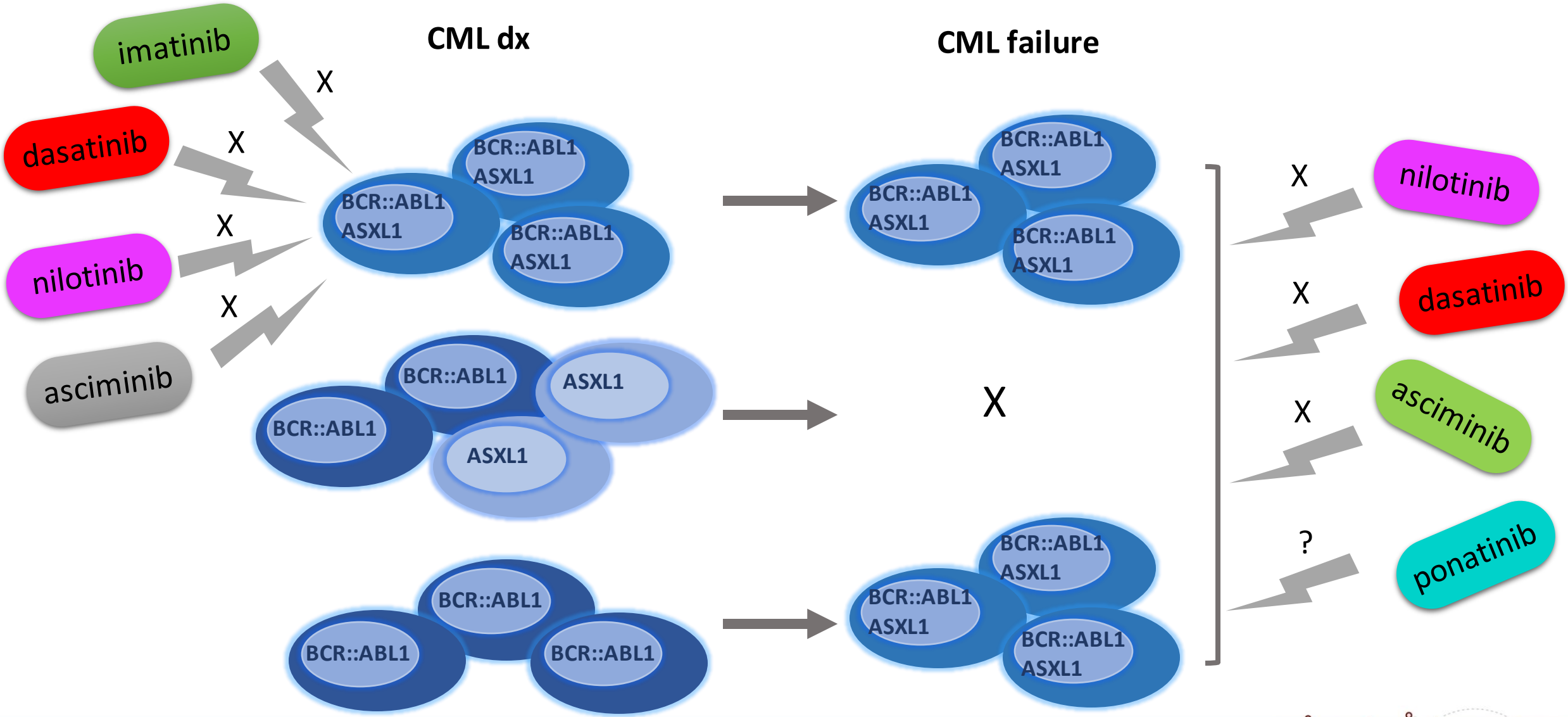


Alox5 reported to be a critical regulator for leukemia stem cells (LSCs) in BCR-ABL-induced chronic myeloid leukemia (**CML**) (Chen et al, Nat Genet 2009)

ALOX5: arachidonate 5-lipoxygenase
BLTR: leukotriene B4 receptor

Miyashita et al, Cancer Sci 2025

How actionable cancer gene/ASXL1 mutations are?



The CML story continues to unfold..
Some unmet needs and open issues

- Fill the gaps in our knowledge about mutations conferring resistance to asciminib
- Explore treatment options against compound mutations
- Investigate the clinical actionability of ASXL1 mutations
- Devise how to best place and integrate NGS and ddPCR for BCR::ABL1 and non-BCR::ABL1 mutation testing